Part VII - Summary

Request for Authorization of genetically modified herbicide tolerant

MS11 x RF3 *Brassica napus*

for food and feed uses, and import and processing, in accordance with articles 5 and 17 of Regulation (EC) N° 1829/2003

EFSA-GMO-NL-2017-XXX

Version CC1

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PART VII – SUMMARY

EFSA-GMO-NL-2017-XXX (MS11 X RF3 *BRASSICA NAPUS*)

1. GENERAL INFORMATION

1.1. Details of application

(a) Member State of application

Netherlands

(b) Application number

EFSA-GMO-NL-2017-XXX

(c) Name of the product (commercial and any other names)

MS11 x RF3 *Brassica napus*

(d) Date of acknowledgement of valid application

Not applicable at the time of submission

1.2. Applicant

(a) Name of applicant

Bayer CropScience LP

(b) Address of applicant

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P.O. Box 12014
Research Triangle Park
RTP, North Carolina 27709
USA

Represented by:
Bayer CropScience N.V.
J.E. Mommaertslaan 14
1831 Diegem
Belgium

(c) Name and address of the representative of the applicant established in the Union (if the applicant is not established in the Union)

Bayer CropScience SA-NV is the contact for this submission and all correspondence should be directed to:

CropScience Division
Bayer CropScience SA-NV
Regulatory Affairs
Square de Meeûs 40
1000 Brussel,
Belgium

1.3. Scope of the application

(a) Genetically modified food

☑ Food containing or consisting of genetically modified plants
☑ Food produced from genetically modified plants or containing ingredients produced from genetically modified plants

(b) Genetically modified feed

☑ Feed containing or consisting of genetically modified plants
☑ Feed produced from genetically modified plants

(c) Genetically modified plants for food or feed uses

☑ Products other than food and feed containing or consisting of genetically modified plants with the exception of cultivation
☐ Seeds and plant propagating material for cultivation in the Union

1.4. Is the product or the uses of the associated plant protection product(s) already authorised or subject to another authorisation within the Union?

No ☑

Yes ☐ (in that case, specify)

Has the genetically modified plant been notified under Part B of Directive 2001/18/EC?

Yes ☐

No ☑ (in that case, provide risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC)

This application requests authorization for food and feed uses, and import and processing and does not include cultivation in the EU. Risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC is provided in the application.

1.5. Has the genetically modified plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC?

No ☑

Yes ☐ (in that case, specify)

1.6. Has the product been subject to an application and/or authorised in a third country either previously or simultaneously to this application?

No ☐

Yes ☑ in that case, specify the third country, the date of application and, where available, a copy of the risk assessment conclusions, the date of the authorisation and the scope of the application

The request for authorization of MS11 x RF3 *B. napus* for import has been submitted in:

Korea

- Ministry of Food and Drug Safety – 8/9/2016
- Rural Development Administration – 26/10/2016

Taiwan

- Food and Drug Administration – 3/10/2016
- Council of Agriculture – 3/30/2017

The request for authorization of MS11 x RF3 *B. napus* for cultivation has been submitted in:

Canada

- Canadian Food Inspection Agency – 26/1/2017
- Health Canada – 26/1/2017

USA

- United States Department of Agriculture – 16/8/2016
- Food and Drug Administration – 26/8/2016

Australia

- Food Standards Australia New Zealand – 1/12/2016

1.7. General description of the product

(a) Name of the recipient or parental plant and the intended function of the genetic modification.

MS11 ***Brassica napus*** (*B. napus*) (male sterile line) was produced by means of *Agrobacterium* mediated transformation using the vector pTCO113. MS11 *B. napus* contains the *barnase* gene (origin *Bacillus amyloliquefaciens*) coding for a ribonuclease, Barnase. The *barnase* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of Barnase in the tapetum cells of MS11 *B. napus* results in lack of viable pollen and male sterility. MS11 *B. napus* contains the *barstar* gene (origin *Bacillus amyloliquefaciens*) coding for the Barstar protein, which is an inhibitor of the Barnase protein. This prophylactic *barstar* gene, driven by the Pnos promoter, is included to enhance transformation frequency. MS11 *B. napus* also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyltransferase (PAT/*bar*) conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant.

RF3 ***B. napus*** (restorer of fertility line) was produced by means of *Agrobacterium*-mediated transformation using vector pTHW118. RF3 *B. napus* contains the *barstar* gene (origin *Bacillus amyloliquefaciens*), coding for the Barstar protein, which is an inhibitor of the Barnase protein. The *barstar* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of the Barstar protein in the tapetum cells leads to restoration of fertility after crossing to a male sterile (MS) *B. napus* line. RF3 *B. napus* also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyl transferase (PAT/*bar*) conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant.

MS11 x RF3 *B. napus* is a stacked product generated through conventional breeding of MS11 *B. napus* and RF3 *B. napus*. No new genetic modification was introduced in the MS11 x RF3 *B. napus*. MS11 x RF3 *B. napus* plants are fully fertile hybrids and express the PAT/*bar* protein which confers tolerance to glufosinate-ammonium.

(b) Types of products planned to be placed on the market according to the authorisation applied for and any specific form in which the product must not be placed on the market (such as seeds, cut-flowers, vegetative parts) as a proposed condition of the authorisation applied for.

The scope of the current application is for authorisation of MS11 x RF3 *B. napus* for import, processing and all uses as any other oilseed rape in the EU, according to Art 3(1) and 15(1) of Regulation (EC) No 1829/2003, with the exception of cultivation. The range of uses of this oilseed rape will be identical to the full range of equivalent uses of conventional oilseed rape.

(c) Intended use of the product and types of users.

MS11 x RF3 *B. napus* will be traded and used in the EU in the same manner as current conventional commercial oilseed rape and by the same operators currently involved in the trade and use of oilseed rape.

(d) Any specific instructions and recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for.

With the exception of the herbicide tolerance, which only has agronomic relevance, the characteristics of MS11 x RF3 *B. napus* oilseed rape and products derived from it are comparable to those of its conventional counterpart and the commercial reference varieties with a history of safe use. Therefore, MS11 x RF3 *B. napus* and its derived products will be stored, packaged, transported, handled and used in the same manner as current commercial oilseed rape products. No specific instructions and/or recommendations are warranted or required for the placing on the market of MS11 x RF3 *B. napus* for import, processing and all uses, excluding cultivation, in the EU.

(e) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for.

MS11 x RF3 *B. napus* is suitable for use throughout the EU as any other oilseed rape. The scope of this application covers the import, processing and all uses of MS11 x RF3 *B. napus*, excluding cultivation.

(f) Any type of environment to which the product is unsuited.

MS11 x RF3 *B. napus* is suitable for use throughout the EU as any other oilseed rape. The scope of this application covers the import, processing and all uses of MS11 x RF3 *B. napus*, excluding cultivation.

(g) Any proposed packaging requirements.

With the exception of the herbicide tolerance, which only has agronomic relevance, the characteristics of MS11 x RF3 *B. napus* are not different from those of its conventional counterpart. Therefore, MS11 x RF3 *B. napus* and derived products will be used in the same manner as other oilseed rape and no specific packaging is required.

(h) Any proposed labelling requirements in addition to those required by other applicable EU legislation then (EC) No 1829/2003 and when necessary a proposal for specific labelling in accordance with Article 13(2) and (3), Article 25(2)(c) and

(d) and Article 25(3) of Regulation (EC) No 1829/2003. In the case of products other than food and feed containing or consisting of genetically modified plants, a proposal for labelling which complies with the requirements of point A(8) of Annex IV to Directive 2001/18/EC must be included.

In accordance with Regulations (EC) No 1829/2003 and 1830/2003, a labelling threshold of 0.9% is applied for the placing on the market of MS11 x RF3 *B. napus* and derived products.

Operators shall be required to label products containing or consisting of MS11 x RF3 *B. napus* with the words "genetically modified oilseed rape" or "contains genetically modified oilseed rape" and shall be required to declare the unique identifier in the list of GMOs that have been used to constitute the mixture that contains or consists of this GMO.

Operators shall be required to label foods and feeds derived from MS11 x RF3 *B. napus* with the words "produced from genetically modified oilseed rape". In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the food or feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using MS11 x RF3 *B. napus* and derived foods and feeds in the EU shall be required to be aware of the legal obligations regarding traceability and labelling of these products. Given that explicit requirements for the traceability and labelling of GMOs and derived foods and feeds are laid down in Regulations (EC) No 1829/2003 and 1830/2003 and that authorised foods and feeds shall be entered in the EU Register for genetically modified food and feed, operators in the food/feed chain will be fully aware of the traceability and labelling requirements for MS11 x RF3 *B. napus*. Therefore, no further specific measures are to be taken by the applicant.

(i) Estimated potential demand

(i) In the EU

There are no anticipated changes to the demand as a result of the introduction of MS11 x RF3 *B. napus* into the oilseed rape as the changes have only an agronomic benefit. It is anticipated that the introduction of MS11 x RF3 *B. napus* will replace some of the oilseed rape in existing food and feed products.

(ii) In EU export markets

There are no anticipated changes to the extent of oilseed rape production in export markets as a result of the introduction of MS11 x RF3 *B. napus* oilseed rape. It is anticipated that the introduction of MS11 x RF3 *B. napus* will replace some of the oilseed rape products.

(j) Unique identifier in accordance with Regulation (EC) No 65/2004

The OECD unique identifier for MS11 x RF3 *B. napus* is BCS-BNØ12-7 x ACS-BNØØ3-6.

1.8. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for its disposal and treatment

Because this application is for consent to import, process and all uses of MS11 x RF3 *B. napus* as any other oilseed rape, not including the cultivation of varieties of MS11 x RF3 *B. napus* in the EU, the only potential means of environmental release would be more likely to occur during import, storage and processing of MS11 x RF3 *B. napus*. However, modern methods of oilseed rape handling minimize losses of seed, so there is little chance of germination of spilled oilseed rape resulting in the development of mature

MS11 x RF3 *B. napus* plants in the EU. Moreover, in the event of incidental spillage, the establishment of volunteer plants would be unlikely, since MS11 x RF3 *B. napus*, like any other oilseed rape, is unlikely to effectively compete with perennial vegetation outside agricultural fields. The likelihood for spilled seed to survive and establish is negligible. Oilseed rape plants outside agricultural fields can produce seed but this is often prevented because most plants do not survive to reach maturity. This is due to competition from other vegetation, management operations such as roadside mowing, the use of broadleaf herbicides, animal predation, diseases and environmental conditions.

MS11 x RF3 *B. napus* is not different in composition, nutritional and agronomic characteristics relative to conventional oilseed rape, except for the introduced tolerance to glufosinate, and therefore, it is unlikely to pose any threat to the EU environment or to require special measures for its containment. Furthermore, oilseed rape volunteers can be easily controlled using currently available selective herbicides (other than glufosinate) or by mechanical means. Therefore, no special measures are considered to be required in case of misuse or unintended release.

2. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

2.1. Complete name

(a) Family name
Cruciferae

(b) Genus
Brassica

(c) Species
napus

(d) Subspecies
oleifera

(e) Cultivar/breeding line
various

(f) Common name
oilseed rape (known as canola in Canada)

2.2. Geographical distribution and cultivation of the plant, including the distribution within the Union

Brassica napus is thought to have originated in the Mediterranean. It was cultivated by ancient civilizations in Asia and the Mediterranean and its oil was used for lighting. It was reportedly grown in Europe for lamp oil and lubrication in the 13th century and in Asia for cooking oil for thousands of years. Oilseed rape became widespread as a source of food and animal feed only after 1960 when Canadian scientists created the first double-low (low-erucic acid and low glucosinolate) variety.

In Europe the main producers of *B. napus* are France, Germany, Ukraine, Poland and United Kingdom. Besides Europe, it is currently grown in Canada, China, India, Pakistan, Australia and the USA.

2.3. Information concerning reproduction (for environmental safety aspects)

(a) Mode(s) of reproduction

Under natural conditions, oilseed rape reproduction is through seeds. Oilseed rape flowers are bisexual and contain six stamens, a pistil of two carpels and a superior ovary. Oilseed rape has the capability of both self- and cross- pollination via both insect and wind. However, the majority of fertilization occurs by self-pollination as the large amounts of pollen produced from each flower out competes the pollen from adjacent flowers. Oilseed rape produces a large amount of pollen which can remain viable for four to five days under field conditions.

(b) Specific factors affecting reproduction

The optimum temperature for vegetative growth of oilseed rape is about 20°C. Reproduction of spring oilseed rape is favoured by dry weather conditions, which favours the activity of insect pollinators, and shorter growing seasons. Winter varieties take advantage of longer growing seasons. Water availability is also of importance, particularly during the period of seed ripening.

(c) Generation time

The generation time in agronomic ecosystems is normally about 4 - 5 months for spring sown crops or 10 - 11 months for autumn sown crops.

2.4. Sexual compatibility with other cultivated or wild plant species (for environmental safety aspects)

Successful hybrid formation depends not only on the sexual compatibility between the plants (whether the same or related species) but the two plants must flower simultaneously, share the same insect pollinator (if insect pollinated) and be sufficiently nearby for the transfer of viable pollen. The consequences of successful transfer will depend on the sexual fertility of the hybrid progeny, vigour and the fertility of subsequent generations or their ability to propagate vegetatively. Given the male sterility of MS11 *B. napus*, the frequency of gene flow from oilseed rape to wild relatives under natural conditions is considered very low. Finally, the fitness of the interspecific hybrids is generally reduced compared to the parents and the stable introgression of a new trait in the weed species genome is confirmed to be extremely difficult.

2.5. Survivability (for environmental safety aspects)

(a) Ability to form structures for survival or dormancy

Oilseed rape is an annual plant that survives through seed formation. If seeds are buried due to e.g. cultivation, they may persist for periods of up to ten years under ideal conditions.

(b) Specific factors affecting survivability

Optimal germination conditions for oilseed rape are 20°C, high water availability (*e.g.* -0.2 MPa water pressure) and exposure to light. Consequently, the greatest proportion of oilseed rape plants that germinates after harvest (‘volunteers’) emerges in response to

tillage. As most of the oilseed rape seeds that fall on the ground after harvesting will still germinate before the winter season, these seedlings will be destroyed by winter conditions. Seeds that get buried deeper can be lost from the seed bank by predation and decay.

2.6. Dissemination (for environmental safety aspects)

(a) Ways and extent of dissemination

Pollen dissemination is mainly affected by wind and insects. Pollinating insects, in particular honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) play a major role in *Brassica napus* pollination. The dynamics of bee-mediated pollen movement depend on the quantity of pollen available (size and density of donor population) and the size and location of the receiving populations, as well as environmental conditions and insect activity

(b) Specific factors affecting dissemination

There is no specific factor affecting seed dissemination (oilseed rape seeds have no special adaptations to encourage transport). The seeds are small and birds and small mammals usually eat them on the spot rather than carrying them away

2.7. Geographical distribution within the Union of the sexually compatible species (for environmental safety aspects)

B. napus can be subdivided into winter and spring forms. The winter annual is grown in regions where winter conditions do not result in very low temperatures. In North America and northern Europe, the spring biotype of *B. napus* is grown that requires no vernalisation prior to flowering.

The main four compatible species of *B. napus* (*Brassica rapa*, *Brassica juncea*, *Hirschfeldia incana*, *Raphanus raphanistrum*) are found throughout Europe, with *Hirschfeldia incana* primarily found in Southern Europe. However, the frequency of gene flow from oilseed rape to these wild relatives under natural conditions is considered very low and the fitness of the interspecific hybrids is generally reduced compared to the parents. Therefore, stable introgression of a new trait in the weed species genome is confirmed to be extremely difficult.

2.8. In the case of plant species not normally grown in the Union, description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts (for environmental safety aspects)

Not relevant as oilseed rape is normally cultivated as a crop in the EU.

2.9. Other potential interactions, relevant to the genetically modified plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms (for environmental safety aspects)

The scope of this application does not include cultivation of MS11 x RF3 *B. napus* seeds in the EU and therefore no potential interactions with organisms in the ecosystem in the EU are expected. However (and in regions where MS11 x RF3 *B. napus* seed products will

be cultivated) (e.g. North America), numerous insects, fungi, mycoplasmas and viruses are pathogenic to *Brassica napus* and attack the crop during the growing season.

3. MOLECULAR CHARACTERISATION

3.1. Information relating to the genetic modification

(a) Description of the methods used for the genetic modification

MS11 x RF3 *B. napus* is a stacked product generated through conventional breeding of MS11 *B. napus* and RF3 *B. napus*. No new genetic modification was introduced in the MS11 x RF3 *B. napus*.

(b) Nature and source of the vector used

MS11 x RF3 *B. napus* is a stacked product generated through conventional breeding of MS11 *B. napus* and RF3 *B. napus*. No new genetic modification was introduced in the MS11 x RF3 *B. napus*.

For MS11, the plasmid vector used was pTCO113, derived from pGSC1700.

For RF3, the plasmid vector used was pTHW118, derived from pGSC1700.

(c) Source of donor nucleic acid(s) used for the transformation, size and intended function of each constituent fragment of the region intended for insertion

MS11 x RF3 *B. napus* is a stacked product generated through conventional breeding of MS11 *B. napus* and RF3 *B. napus*. No new genetic modification was introduced in the MS11 x RF3 *B. napus*. The DNA inserts in MS11 x RF3 *B. napus* are inherited from MS11 and RF3. Information on the genetic elements in MS11 *B. napus* and RF3 *B. napus* are provided below.

Size, source and intended function of each constituent component of the inserted DNA fragment inherited from MS11 *B. napus*

Nt Positions	Orientation	Origin
1 - 25		**RB:** right border region of the T-DNA of *Agrobacterium tumefaciens* (Zambryski, 1988 M-234499-01-2)
26 - 97		Polylinker sequences: sequence used in cloning
98 - 309	Counter clockwise	**3'g7:** 3´ untranslated region of the TL-DNA gene 7 of the *Agrobacterium tumefaciens* octopine Ti plasmid (Dhaese et al., 1983 M-180190-01-1)
310 - 331		Polylinker sequences: sequence used in cloning
332 - 883	Counter clockwise	***bar:*** coding sequence of the phosphinothricin acetyltransferase gene of *Streptomyces hygroscopicus* (Thompson et al., 1987 M-122742-01-1).
884 - 2613	Counter clockwise	**PssuAt:** promoter region of the ribulose-1,5-biphosphate carboxylase small subunit gene of *Arabidopsis thaliana* (Krebbers et al., 1988 M-180191-01-1)

Nt Positions	Orientation	Origin
2614 - 2658		Polylinker sequences: sequence used in cloning
2659 - 2919	Counter clockwise	**3'nos:** 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (Depicker et al., 1982 M-131630-01-2)
2920 - 2935		Polylinker sequences: sequence used in cloning
2936 - 3033	Counter clockwise	**3'barnase:** 3´ untranslated region of the barnase gene from *Bacillus amyloliquefaciens* (Hartley, 1988 M-180195-01-1)
3034 - 3369	Counter clockwise	***barnase:*** coding sequence of the barnase gene of *Bacillus amyloliquefaciens* (Hartley, 1988 M-180195-01-1)
3370 - 3371		Polylinker sequences: sequence used in cloning
3372 - 4879	Counter clockwise	**Pta29:** promoter of the anther-specific gene TA29 of *Nicotiana tabacum* (tobacco) (Seurinck et al., 1990 M-180196-01-1)
4880 - 4920		Polylinker sequences: sequence used in cloning
4921 - 5214	Clockwise	**Pnos:** promoter region of the nopaline synthase gene of *Agrobacterium tumefaciens* (Depicker et al., 1982 M-131630-01-2)
5215 - 5216		Polylinker sequences: sequence used in cloning
5217 - 5489	Clockwise	***barstar:*** coding sequence of the barstar gene of *Bacillus amyloliquefaciens* (Hartley, 1988 M-180195-01-1)
5490 - 5554		Polylinker sequences: sequence used in cloning
5555 - 5766	Clockwise	**3'g7:** 3´ untranslated region of the TL-DNA gene 7 of the *Agrobacterium tumefaciens* octopine Ti plasmid (Dhaese et al., 1983 M-180190-01-1)
5767 - 5840		Polylinker sequences: sequence used in cloning
5841 - 5865		**LB:** left border region of the T-DNA of *Agrobacterium tumefaciens* (Zambryski, 1988 M-234499-01-2)
5866 - 7745	Counter clockwise	***aadA*:** fragment including the aminoglycoside adenyltransferase gene of *Escherichia coli* (Fling et al., 1985 M-231609-01-1)
7746 - 8181	Counter clockwise	***barstar:*** fragment including the barstar gene of *Bacillus amyloliquefaciens* (Hartley, 1988 M-180195-01-1)
8182 - 8405	Counter clockwise	***aadA*:** fragment including the residual upstream sequences of the aminoglycoside adenyltransferase gene of *Escherichia coli* (Fling et al., 1985 M-231609-01-1)
8406 - 12177		**ORI pVS1:** fragment including the origin of replication of the plasmid pVS1 of *Pseudomonas aeruginosa* (Heeb et al., 2000 M-453202-01-1)

Nt Positions	Orientation	Origin
12178 - 13540		**ORI ColE1:** fragment including the origin of replication from the plasmid pBR322 for replication in *Escherichia coli* (Bolivar et al., 1977 M-147993-01-1).

Size, source and intended function of each constituent component of the inserted DNA fragment inherited from RF3 *B. napus*

Definition	Source	Size (bp)	Function
Right border sequence	*A. tumefaciens*	25	T-DNA integration
Polylinker sequence	Synthetic	28	Plasmid cloning
TL-DNA sequence	*A. tumefaciens*	37	None
Polylinker sequence	Synthetic	7	Plasmid cloning
Terminating signal from TL-DNA gene 7	*A. tumefaciens*	212	Stop signal
Polylinker sequence	Synthetic	21	Plasmid cloning
Glufosinate tolerance gene	*S. hygroscopicus*	552	Selectable marker and herbicide tolerance
Promoter	*A. thaliana*	1726	Constitutive promoter targeting expression mainly to green tissue
Polylinker sequence	Synthetic	50	Plasmid cloning
Polyadenylation region of nopaline synthase gene	*A. tumefaciens*	261	Stop signal
Polylinker sequence	Synthetic	21	Plasmid cloning
Terminating signal of *barstar* gene	*B. amyloliquefaciens*	40	Stop signal
Ribonuclease inhibitor gene	*B. amyloliquefaciens*	273	Fertility Restoration
Promoter	*N. tabacum*	1510	Expression only in anthers
Polylinker sequence	Synthetic	45	Plasmid cloning
Left border sequence	*A. tumefaciens*	25	T-DNA integration

3.2. Information relating to the genetically modified plant

MS11 *Brassica napus* (*B. napus*) (male sterile line) was produced by means of *Agrobacterium* mediated transformation using the vector pTCO113. MS11 *B. napus* contains the *barnase* gene (origin *Bacillus amyloliquefaciens*) coding for a ribonuclease, Barnase. The *barnase* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of Barnase in the tapetum cells of MS11 *B. napus* results in lack of viable pollen and male sterility. MS11 *B. napus* contains the *barstar* gene (origin *Bacillus amyloliquefaciens*) coding for the Barstar protein, which is an inhibitor of the Barnase protein. This prophylactic *barstar* gene, driven by the Pnos promoter, is included to enhance transformation frequency. MS11 *B. napus* also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyltransferase (PAT/*bar*) conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant.

RF3 *B. napus* (restorer of fertility line) was produced by means of *Agrobacterium*-mediated transformation using vector pTHW118. RF3 *B. napus* contains the *barstar* gene (origin *Bacillus amyloliquefaciens*), coding for the Barstar protein, which is an inhibitor of the Barnase protein. The *barstar* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of the Barstar protein in the tapetum cells leads to restoration of fertility after crossing to a MS *B. napus* line. RF3 *B. napus* also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyl transferase (PAT/*bar*) conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant.

MS11 x RF3 *B. napus* is a stacked product generated through conventional breeding of MS11 *B. napus* and RF3 *B. napus*. No new genetic modification was introduced in the MS11 x RF3 *B. napus*. MS11 x RF3 *B. napus* plants are fully fertile hybrids and express the PAT/*bar* protein which confers tolerance to glufosinate-ammonium.

3.2.1. Description of the trait(s) and characteristics which have been introduced or modified

The *barnase* and *barstar* gene and hybrid system

MS11 *B. napus* contains the *barnase* gene coding for a ribonuclease, Barnase. The barnase gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of Barnase in the tapetum cells of MS11 *B. napus* results in lack of viable pollen and male sterility. MS11 *B. napus* contains the *barstar* gene coding for the Barstar protein, which is an inhibitor of the Barnase protein. This prophylactic *barstar* gene, driven by the Pnos promoter, is included to enhance transformation frequency. RF3 *B. napus* contains the *barstar* gene, coding for the Barstar protein, which is an inhibitor of the Barnase protein. The *barstar* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of the Barstar protein in the tapetum cells leads to restoration of fertility after crossing to a MS *B. napus* line. MS11 x RF3 *B. napus* is a stacked product generated through conventional breeding of MS11 *B. napus* and RF3 *B. napus*. MS11 x RF3 *B. napus* plants are fully fertile hybrids.

The *bar* gene and tolerance to glufosinate-ammonium

Both MS11 and RF3 *B. napus* contain the *bar* gene coding for phosphinothricin acetyltransferase (PAT/*bar*) conferring tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant. The *bar* gene has been isolated from *Streptomyces hygroscopicus*, a microorganism that produces bialaphos. Bialaphos or its synthetically produced component glufosinate-ammonium is classified as herbicide with phosphinothricin as the active ingredient. Phosphinothricin acts by the inhibition of a specific amino acid biosynthesis pathway in plants. It is a potent inhibitor of glutamine synthetase (GS), an enzyme that plays a central role in the assimilation of ammonia and in the regulation of the nitrogen metabolism in the plant. Phosphinothricin based herbicides are highly effective against plants, but are safe to humans and animals and are rapidly biodegraded in the environment. The *bar* gene product, PAT, metabolizes phosphinothricin to an inactive, acetylated derivative.

Glufosinate ammonium is defined as a non-selective and partially systemic contact herbicide. After application, the active ingredient phosphinothricin acts via the leaf. No action via the roots could be detected in plants after emergence and no damage is caused to seedlings before emergence. Shortly after the uptake, the herbicide will disturb the ammonium metabolism of the treated plants. The systemic transport from treated leaves to other

parts of the plant is nevertheless limited. Ammonia is an important link between catabolic and anabolic processes in the plant metabolism and it is released and re-assimilated in large amounts at different processes. Regardless of the origin, however, it is essential that the ammonia is rapidly converted into a form that is not toxic to the organism. This detoxifying reaction is guided by the glutamine synthetase enzyme.

3.2.2. Information on the nucleic acid(s) sequences actually inserted or deleted

(a) The copy number of all detectable inserts, both complete and partial

Southern blot analysis of MS11 x RF3 *B. napus* confirmed the presence of MS11 and RF3 DNA inserts in MS11 x RF3 *B. napus*.

(b) In case of deletion(s), size and function of the deleted region(s)

Not applicable. MS11 x RF3 *B. napus* was developed by crossing the single parental lines MS11 *B. napus* and RF3 *B. napus* using traditional breeding methods.

(c) Subcellular location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

As supported by the Southern blot analysis of MS11 x RF3 *B. napus*, the inserted DNA fragments from the parental lines MS11 *B. napus* and RF3 *B. napus* are inherited in MS11 x RF3 *B. napus*.

(d) The organisation of the inserted genetic material at the insertion site

Since the inserts in the single events MS11 and RF3 were retained in MS11 x RF3 *B. napus*, the characteristics of the insertions and the 5' and 3' flanking sequences should be conserved in MS11 x RF3 *B. napus*.

(e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification, as well as direct changes in expression of genes as a result of the modification

Not applicable

3.2.3. Information on the expression of the insert

(a) Information on developmental expression of the insert during the life cycle of the plant

The expression of the Barnase, Barstar and PAT/*bar* proteins were analysed in different tissues of MS11 x RF3 *B. napus* collected at different developmental stages. The proteins were quantified in whole plant, root, raceme, and grain (seed) matrices. The values of the Barnase, Barstar and PAT/*bar* proteins in MS11 x RF3 *B. napus* were compared with the corresponding values in the *B. napus* parental events.

Based on the observed overlapping ranges of measured analyte concentrations, it appears that Barnase, Barstar and PAT/*bar* are similarly expressed when comparing MS11 x RF3 *B. napus* to MS11 *B. napus* and RF3 *B. napus*. Therefore, it can be concluded that there are no indications for interactions between the single events in MS11 x RF3 *B. napus.*

(b) Parts of the plant where the insert is expressed

The Barnase, Barstar and PAT/*bar* proteins were quantified in whole plant, root, raceme, and

grain (seed) matrices. In this application, the expression levels in seeds are discussed, since seed is the part of the *B. napus* plant relevant for the scope of this application. The expression level of the Barnase, Barstar and PAT/*bar* proteins was determined in MS11 x RF3 *B. napus* seeds harvested from field grown plants in Canada and the USA.

3.2.4. Genetic stability of the insert and phenotypic stability of the genetically modified plant

The results of the Southern blot analysis of MS11 x RF3 *B. napus* demonstrated the stability of the inserted sequences of MS11 and RF3 in MS11 x RF3 *B. napus*, and confirmed that no detectable rearrangements of these inserts occurred.

The results of the analysis of Barnase, Barstar and PAT/*bar* proteins in MS11 x RF3 *B. napus* showing comparable levels to the expression in the single parental lines MS11 *B. napus* and RF3 *B. napus* and confirmed the phenotypic stability of MS11 x RF3 *B. napus*.

3.2.5. Information (for environmental safety aspects) on how the genetically modified plant differs from the recipient plant in

(a) Mode(s) and /or rate of reproduction

Results of these field trials showed that there are no unexpected biologically relevant changes in the agronomic and phenotypic characteristics of MS11 x RF3 *B. napus* compared to the conventional counterpart, taking into account natural variation. On the basis of these studies, it is possible to conclude that no differences in the mode or rate of reproduction, dissemination, survivability or other agronomic, phenotypic or ecological characteristics are expected in MS11 x RF3 *B. napus* and that MS11 x RF3 *B. napus* is not different in its phenotypic and agronomic behaviour relative to conventional oilseed rape, except for the introduced trait.

(b) Dissemination

No differences in the dissemination compared to the conventional counterpart have been observed in agronomic and phenotypic assessments conducted with MS11 x RF3 *B. napus*.

(c) Survivability

No differences in the survivability compared to the conventional counterpart have been observed in agronomic assessments conducted with MS11 x RF3 *B. napus*.

(d) Other differences

Except for the introduced trait that is of agronomic interest, the agronomic assessments in the field did not reveal any biologically relevant differences between MS11 x RF3 *B. napus* and its conventional counterpart.

3.2.6. Any change to the ability of the genetically modified plant to transfer genetic material to other organisms (for environmental safety aspects)

(a) Plant to bacteria gene transfer

MS11 x RF3 *B. napus* was developed by crossing the single parental lines MS11 *B. napus* and RF3 *B. napus* using traditional breeding methods. Therefore the inserted DNA fragments from the parental lines are inherited in MS11 x RF3 *B. napus*. The inserted sequences in the single events are not providing different abilities to transfer genetic

material compared to conventional *B. napus* and no other elements in the inserts suggest that there could be an increase of the probability of homologous recombination. The stacking of the single events using conventional breeding techniques has not resulted in any alteration on the inserts in MS11 x RF3 *B. napus*. Therefore, the likelihood that plant to bacteria gene transfer occurs is highly unlikely.

(b) Plant to plant gene transfer

Based on the observation that reproductive morphology in MS11 x RF3 *B. napus* is unchanged compared to conventional oilseed rape, the out-crossing frequency to other oilseed rape varieties or to wild relatives would be unlikely to be different for MS11 x RF3 *B. napus*, when compared to conventional oilseed rape varieties. Furthermore, the scope of the current application does not include the cultivation of MS11 x RF3 *B. napus* varieties in the EU. As a consequence exposure to the environment will be very limited.

4. COMPARATIVE ANALYSIS

4.1. Choice of the conventional counterpart and additional comparators

The non-GM conventional counterpart, N90-740, was included for comparative purposes, because it was the original genetic background selected for transformation of the MS11 x RF3 *B. napus* and is appropriate to be grown in the *B. napus* growing regions in Canada and USA. The MS11 x RF3 *B. napus* and its conventional counterpart have the same genetic background, *B. napus* N90-740.

Six non-GM commercial *B. napus* reference varieties were included to provide reference ranges for the comparative assessment of the agronomic and composition data. The selected *B. napus* varieties cover several germplasm pools.

4.2. Experimental design and statistical analysis of data from field trials for comparative analysis

The *B. napus* plants were grown in 2014 at sites representative of commercial *B. napus* production areas in Canada and the USA.

An analysis of variance (ANOVA) was conducted in a combined-site analysis in which the data was pooled across all sites. ANOVA models were used to perform difference and equivalence tests according to the 2010 EFSA Scientific opinion on statistical considerations for the safety evaluation of GMOs.

4.3. Selection of material and compounds for analysis

The key nutrients and other nutritionally important components that were selected for analysis of MS11 x RF3 *B. napus* in the compositional study were chosen on the basis of internationally accepted guidance provided by the OECD consensus document on compositional considerations for new varieties of oilseed rape.

4.4. Comparative analysis of agronomic and phenotypic characteristics

An assessment of the phenotypic and agronomic characteristics of MS11 x RF3 *B. napus* compared to conventional oilseed rape has been performed in the field. Results of this field study showed that there are no unexpected and biologically relevant changes in the

agronomic or phenotypic characteristics of MS11 x RF3 *B. napus* compared to the oilseed conventional counterpart, taking into account natural variation.

4.5. Effect of processing

The effects of processing on MS11 x RF3 *B. napus* are not expected to be different from the effects on conventional *B. napus*.

MS11 x RF3 *B. napus* is not different from conventional *B. napus*, except for the expressed Barnase, Barstar and PAT/*bar* proteins. During processing, proteins are subjected to harsh conditions that drastically change the physical forces leading to denaturation and loss of protein function. Processing using heat, for example cooking, high pressure steam, plus solvents and alkali treatments, will degrade the Barnase, Barstar and PAT/*bar* proteins. Thus, dietary exposure to functionally active proteins in processed food products can be negligible. Therefore, MS11 x RF3 *B. napus* and its derived food and feed products are highly unlikely to be different from the equivalent foods and feeds from conventional *B. napus* and as a consequence, toxicological tests with MS11 x RF3 *B. napus* processed products are not scientifically justified.

5. TOXICOLOGY

(a) Toxicological testing of the newly expressed proteins

MS11 x RF3 *B. napus* expresses the proteins Barnase, Barstar and PAT/*bar*. These three proteins have very specific activities in MS11 x RF3 *B. napus*.

The available information for the assessment of the newly expressed proteins present in MS11 x RF3 *B. napus* indicates that no adverse effects on human or animal health are expected. Furthermore, in absence of indications of potential interactions between the three newly-expressed proteins as well as between the MS11 and RF3 events, as suggested in the molecular analysis and comparative assessment, the conclusions of the safety assessment for the individual Barnase, Barstar and PAT/*bar* proteins are not changed when their expression in MS11 x RF3 *B. napus* is considered.

(b) Testing of new constituents other than proteins

Not applicable as the genetic modification in MS11 x RF3 *B. napus* does not give rise to the expression of any new constituents other than the Barnase, Barstar and PAT/*bar* proteins. The comparative assessment of MS11 x RF3 *B. napus* showed no biologically relevant differences between MS11 x RF3 *B. napus* and its conventional counterpart and/or lack of equivalence, taking into account natural variation. Therefore, there is no need for further assessment.

(c) Information on natural food and feed constituents

No relevant changes in the composition of MS11 x RF3 *B. napus* were identified, therefore the levels of food and feed constituents in MS11 x RF3 *B. napus* have not been altered and there is no need for further assessment.

(d) Testing of the whole genetically modified food and feed

The molecular characterization of MS11 x RF3 *B. napus* demonstrated the integrity of the inserts in MS11 x RF3 *B. napus* when compared to the single parental lines. The comparative assessment of MS11 x RF3 *B. napus* showed no biologically relevant differences between MS11 x RF3 *B. napus* and its conventional counterpart and/or lack of equivalence, taking into account natural variation. Therefore, there are not indications

of possible interactions between MS11 and RF3 events in MS11 x RF3 *B. napus* and whole food and/or feed testing with MS11 x RF3 *B. napus* is not deemed to be necessary.

From the results of the 90-day feeding study in rats conducted with MS11 *B. napus*, it was concluded that the MS11 *B. napus* meal incorporated up to 15% (w/w) in the diet had no adverse effects on the growth or health of Sprague Dawley rats. The same conclusion has been drawn from the results of the 90-day feeding study in rats conducted with RF3 *B. napus* meal incorporated up to 15% (w/w) in the diet of Sprague Dawley rats.

6. ALLERGENICITY

(a) Assessment of allergenicity of the newly expressed protein

The data provided lead to the conclusion that the Barnase, Barstar and PAT/*bar* proteins are unlikely to be allergenic. In addition, there is no evidence that there could be interactions between these three proteins that would lead to additive, synergistic or antagonistic activities. Therefore, it was considered unlikely that potential interactions could change the allergenicity of these proteins in MS11 x RF3 *B. napus*.

(b) Assessment of allergenicity of the whole genetically modified plant

Equivalence of MS11 x RF3 *B. napus* (with the exception of the introduced trait) to the conventional counterpart has been demonstrated on the basis of the compositional analysis. The Barnase, Barstar and PAT/*bar* proteins expressed in MS11 x RF3 *B. napus* are unlikely to be allergenic. Therefore no increased allergenicity is anticipated for MS11 x RF3 *B. napus*.

7. NUTRITIONAL ASSESSMENT

(a) Nutritional assessment of the genetically modified food

The genetic modifications in MS11 x RF3 *B. napus* are not intended to change nutritional characteristics of MS11 x RF3 *B. napus* compared to conventional *B. napus*. Therefore MS11 x RF3 *B. napus* is not expected to be more or less attractive for use as food, so anticipated dietary intake of rapeseed-derived foods is not expected to be changed upon commercialization of MS11 x RF3 *B. napus*.

Compositional analysis demonstrated that MS11 x RF3 *B. napus* is not different from its conventional counterpart (identified differences were found not biologically relevant), except for the introduced traits taking into account natural variation. Therefore, there is no need to carry out further nutritional studies with food derived from MS11 x RF3 *B. napus*.

(b) Nutritional assessment of the genetically modified feed

The genetic modifications in MS11 x RF3 *B. napus* are not intended to change nutritional characteristics of MS11 x RF3 *B. napus* on compared to conventional *B. napus*. Therefore MS11 x RF3 *B. napus* is not expected to be more or less attractive for use as feed.

Compositional analysis demonstrated that MS11 x RF3 *B. napus* is not different from its conventional counterpart (identified differences were found not biologically relevant), except for the introduced traits taking into account natural variation. Therefore, there is no need to carry out further nutritional studies with feed derived from MS11 x RF3 *B. napus*.

8. EXPOSURE ASSESSMENT – ANTICIPATED INTAKE/EXTENT OF USE

MS11 x RF3 *B. napus* was developed by crossing the single *B. napus* parental lines MS11 and RF3 using traditional breeding methods. The intended trait is herbicide tolerance, therefore not intended to modify the nutritional parameters of MS11 x RF3 *B. napus*. MS11 x RF3 *B. napus* is not intended to be processed into products with enhanced functionality. The dietary role of MS11 x RF3 *B. napus* will be the same as non-GM *B. napus*. The use of the food and feed derived from MS11 x RF3 *B. napus* will be the same as food and feed from non-GM *B. napus*. It is expected that the introduction of MS11 x RF3 *B. napus* will replace some of the existing commercial *B. napus*-derived products. Therefore, no change is expected in the consumption of *B. napus* and *B. napus*-derived products.

The exposure assessment in humans and animals indicates that there is minimal dietary exposure to Barnase, Barstar and PAT/*bar* from consumption of foods and feed derived from MS11 x RF3 *B. napus*.

9. RISK CHARACTERISATION

MS11 x RF3 *B. napus* was developed by crossing the single parental lines MS11 *B. napus* and RF3 *B. napus* using traditional breeding methods. No new genetic modification was introduced in MS11 x RF3 *B. napus*. MS11 *B. napus* and RF3 *B. napus* have been previously assessed in applications EFSA-GMO-BE-2016-138 and EFSA-GMO-RX-MS8xRF3, respectively.

A comprehensive risk characterization of MS11 x RF3 *B. napus* has been carried out by considering all available evidence from the analyses discussed through this application. The following conclusions from molecular characterization, phenotypic and agronomic analyses, compositional analyses, toxicology assessment, allergenicity assessment and exposure assessment have been considered:

- Southern analyses demonstrated that the inserts in the single events MS11 and RF3 were retained in MS11 x RF3 *B. napus*.

  Expression studies showed that the mean Barnase, Barstar and PAT/*bar* protein levels in MS11 x RF3 *B. napus* seeds are comparable to the protein levels in each single event MS11 and RF3. Consequently the combination of the two single parental events does not alter the expression of the inserted genes and therefore there is no evidence of interactions between the inserts. The results of the expression study confirmed the phenotypic stability of MS11 x RF3 *B. napus*.

  Bioinformatics analysis of 5' and 3' flanking regions of MS11 and RF3 did not provide any evidence that functional endogenous genes or ORFs were interrupted upon transformation in MS11 and RF3. In MS11 and RF3 inserted sequences there are neither allergenic nor toxicological *in silico* findings associated with the presence of the putative ORF polypeptides or putative products of predicted genes.

  Based on the above information, no unintended changes and no indications of potential interactions between the single events or between the newly expressed proteins were identified. Therefore it can be concluded that the molecular characterization of MS11 x RF3 *B. napus* does not indicate safety concerns.

- The comparative assessment of MS11 x RF3 *B. napus* showed no differences for the agronomic and phenotypic characteristics and for the *B. napus* seed composition parameters that would require further assessment with respect to their possible impact on food and feed safety and its nutritional properties.

The comparative analysis of the continuous agronomic and phenotypic endpoints showed no statistically significant differences between MS11 x RF3 *B. napus* (conventional herbicide management and treated with the intended herbicide) and its conventional counterpart for any of the endpoints with the exception of Average Plant Height in the comparison between MS11 x RF3 *B. napus* (treated with the intended herbicide) and its conventional counterpart. All continuous agronomic and phenotypic endpoints, including Average Plant Height, were equivalent to the reference varieties.
The comparative analysis of the categorical agronomic and phenotypic endpoints showed no statistically significant differences between MS11 x RF3 *B. napus* plants (conventional herbicide management and treated with the intended herbicide) and the conventional counterpart plants for any of these endpoints. All mean values of the categorical agronomic and phenotypic endpoints were within the minimum to maximum range of the reference varieties.
The comparative analysis of the *B. napus* grain composition data showed no statistically significant differences between MS11 x RF3 *B. napus* (conventional herbicide management) and its conventional counterpart for 27 out of 57 composition parameter for which the data allowed statistical analysis. All 30 composition parameters for which MS11 x RF3 *B. napus* grain (conventional herbicide management) was found to be statistically significantly different to the conventional counterpart were equivalent or more likely equivalent to the reference varieties.
The comparative analysis of the *B. napus* grain composition data showed no statistically significant differences between MS11 x RF3 *B. napus* (treated with the intended herbicide) and its conventional counterpart for 29 out of 57 composition parameter that were statistically analysed. All 28 composition parameters for which MS11 x RF3 *B. napus* grain (conventional herbicide management) was found to be statistically significantly different to the conventional counterpart were equivalent or more likely equivalent to the reference varieties.
The results of four composition parameters that were classified as different and more likely equivalent than not or as not different and less likely equivalent than not (aspartic acid) to the reference varieties were further discussed and the respective mean values were compared with ranges from the OECD Consensus Document for low erucic rapeseed (canola) and from the International Life Sciences Institute Crop Composition Database (ILSI CCDB). The kind and magnitude of the observed differences lack relevance from a biological and nutritional point of view, because statistically significant differences to the conventional counterpart were not detected for both MS11 x RF3 *B. napus* entries, absolute differences between mean values were smaller than the standard deviation of the conventional counterpart, no tendency for the differences were seen between acid detergent fiber mean values of MS11 x RF3 *B. napus* and the conventional counterpart grain, and/or no detrimental effects are expected.

For most of the composition parameters showing differences and/or lack of equivalence, the genotype by site interaction test was not significant and, consequently, the by-site analyses were not evaluated. Significant genotype by site interactions were only found for moisture, neutral detergent fiber, and calcium. The by-site analyses for these three parameters were significant at only a few sites. Hence, the identified differences were not considered biologically relevant.
This analysis established the comparability of MS11 x RF3 *B. napus* to its conventional counterpart and equivalency of MS11 x RF3 *B. napus* to the reference varieties. In conclusion, based on the comparative analysis, there were no unexpected or unintended effects and no impact on either the agronomic performance of the plants or the nutritional value of the grain from MS11 x RF3 *B. napus* plants as a result of the genetic modification of the *B. napus* plants and the combination of MS11 *B. napus* and RF3 *B. napus* by conventional breeding.

- The available information for the assessment of the newly expressed proteins present in MS11 x RF3 *B. napus* indicates that no adverse effects on human or animal health are expected. Furthermore, in the absence of interactions between the three proteins as well as between the MS11 and RF3 events, as suggested by the molecular analysis and comparative assessment, the conclusions of the safety assessment for the individual Barnase, Barstar and PAT/*bar* proteins are not changed when their expression in MS11 x RF3 *B. napus* is considered.

  The results of the comparative assessment conducted on MS11 x RF3 *B. napus* supports a conclusion that no biologically relevant differences, except for the introduced traits, were identified in the composition data obtained from MS11 x RF3 *B. napus* or in its agronomics and phenotypic characteristics that would require further assessment with respect to their possible impact on food and feed safety and nutritional properties. Therefore, there are no indications of any potential adverse effect that could arise from natural constituents changes.

  Based on the results of dietary administration of MS11 *B. napus* meal for at least 90 consecutive days at a concentration of 15% in the diet had no adverse effects on the growth or health of Sprague Dawley rats. Also no adverse effects on the growth or health of Sprague Dawley rats have been reported from dietary administration of RF3 *B. napus* meal for at least 90 consecutive days at a concentration of 15% in the diet.

  The results of the toxicological assessment indicate that consumption of MS11 x RF3 *B. napus* food and feed products will be as safe as consumption of equivalent products from conventional *B. napus*, regardless of the anticipated intake level.

- Bioinformatics analysis demonstrated that there are no biologically relevant sequence similarities to allergens when Barnase, Barstar and PAT/*bar* protein sequences were used as query sequences for a FASTA search against the allergen database.

  There is no evidence that there could be interactions between these three proteins that would lead to additive, synergistic or antagonistic activities. Therefore, it is unlikely that potential interactions could occur that would change the allergenicity of these proteins in MS11 x RF3 *B. napus*.

  Based on the weight of evidence approach it can be concluded that the newly expressed Barnase, Barstar and PAT/*bar* are unlikely to be allergenic.

  The comparative analysis of MS11 x RF3 *B. napus* identified no biologically and nutritionally relevant differences (except for introduced traits) between MS11 x RF3 *B. napus* and its conventional counterpart, taking into account natural variation. The newly expressed proteins are unlikely to be allergenic. Therefore, no increased allergenicity is anticipated for MS11 x RF3 *B. napus* or for the food derived from MS11 x RF3 *B. napus* in comparison to the food derived from the conventional *B. napus* varieties.

- MS11 x RF3 *B. napus* was developed by crossing the single parental lines MS11 and RF3 using traditional breeding methods. As expected, in the comparative assessment of MS11 x RF3 *B. napus* no indications of unintended changes in nutritional value due to the combination of the single parental lines have been observed. Therefore the food and feed derived from MS11 x RF3 *B. napus* is assumed to be nutritionally equivalent to food and feed derived from conventional *B. napus* varieties.

- According to the European Comprehensive Food Consumption Database, the main food product derived from oilseed rape is the rapeseed oil. The processing of rape seeds to rapeseed oil of food grade quality leads to a degradation of all protein compounds. Therefore, the dietary exposure to the PAT/*bar* protein via consumption

of food grade rapeseed oil was considered to be negligible. The European consumers will not be exposed to the PAT/*bar* protein via the consumption of refined rapeseed oil derived from MS11 x RF3 *B. napus* seeds. Therefore, this dietary exposure assessment supports the conclusion that the risk to European consumers from MS11 x RF3 *B. napus* is negligible.

The evidences presented throughout this application and summarized above demonstrate that:

- The food and feed derived from MS11 x RF3 *B. napus* has no adverse effects on human and animal health;
- The food derived from MS11 x RF3 *B. napus* does not differ from the food which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer compared to conventionally produced food;
- The food derived from MS11 x RF3 *B. napus* does not mislead the consumer;
- The feed derived from MS11 x RF3 *B. napus* not differ from the feed which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for animals or humans compared to conventionally produced feed;
- The feed derived from MS11 x RF3 *B. napus* does not harm or mislead the consumer by impairing distinctive features of the animal products compared to conventionally produced feed.

The assumptions made during the risk assessment are very conservative and include the following:

- All *B. napus* grain consumed in the EU would be from MS11 x RF3 *B. napus* plants
- No loss or degradation of protein would occur during processing and food preparation of *B. napus* seed products.

The labelling requirements specified in Articles 5(3)(f) and 17(3)(f) of Regulation (EC) No 1829/2003 are not applicable because the characteristics of the food and feed products from MS11 x RF3 *B. napus* are not different from the characteristics of its conventional counterpart taking into account natural variation.

10. POST-MARKET MONITORING ON GENETICALLY MODIFIED FOOD/FEED

The risk characterization of MS11 x RF3 *B. napus* has shown that the risk for potential adverse effects on human and animal health is negligible in the context of the intended uses of MS11 x RF3 *B. napus*. It is therefore considered that there is no need for post marketing monitoring of food and feed derived from MS11 x RF3 *B. napus*.

11. ENVIRONMENTAL ASSESSMENT

11.1. Mechanism of interaction between the genetically modified plant and target organisms

In this area of assessment, the main environmental concern, according to the EFSA ERA Guidance, is that target organisms develop resistance to the insect or pathogen tolerance traits expressed by the GM plant.

MS11 x RF3 *B. napus* has been developed to confer herbicide tolerance. The scope of this application covers the import, processing and food and feed use of MS11 x RF3 *B. napus* in the EU. According to the EFSA ERA Guidance: "*resistance development is only relevant for applications with scope cultivation of GM plants and not for applications restricted to import and processing of GM plants and their products*". Therefore, an assessment of the potential resistance development in target organisms resulting from the import, processing and food and feed use of MS11 x RF3 *B. napus* is not relevant for this application. Even considering a scenario where accidental spillage of viable material of MS11 x RF3 *B. napus* occurred and some plants grew in the EU, the levels of exposure would be low and limited temporally and spatially. The likelihood of target organisms developing resistance under this scenario would be "highly unlikely" and any consequences on target organism populations would be "marginal", therefore the risk would be "negligible".

11.2. Potential changes in the interactions of the genetically modified plant with the biotic environment resulting from the genetic modification

The scope of the application is for food and feed uses, import and processing and excludes cultivation. The environmental exposure is limited to accidental release of MS11 x RF3 *B. napus* during transportation and processing for food and feed.

(a) Persistence and invasiveness

The persistence and invasiveness of each of the single events present in MS11 x RF3 *B. napus* have been previously assessed. The conclusion was that the genetic modification introduced in each of these events does not alter the persistence and invasiveness characteristics of these single events in the EU. Since the agronomic and phenotypic studies presented in this application also show that MS11 x RF3 *B. napus* does not differ in characteristics indicative of persistence and invasiveness from the conventional crop, it can be concluded that the stacking of MS11 and RF3 using conventional breeding techniques does not result in a stacked trait product with potentially harmful changes in persistence and invasiveness characteristics with respect to the conventional crop.

(b) Selective advantage or disadvantage

Compared with conventional oilseed rape, the introduced herbicide tolerance trait in MS11 x RF3 *B. napus* confer a selective advantage only under specific conditions (i.e. following treatment with trait-specific herbicide). The advantage is of purely agronomic interest and presents negligible risk to the non-agricultural environments. Given the scope of this application, the likelihood is negligible for the inherited traits in MS11 x RF3 *B. napus* to confer any meaningful competitive advantage or disadvantage of relevance to the environment.

(c) Potential for gene transfer

The scope of this application covers the import, processing and all uses of MS11 x RF3 *B. napus* as any other oilseed rape in the EU, excluding cultivation. Therefore, no deliberate release of viable plant material in the EU environment is expected and interactions of MS11 x RF3 *B. napus* with the biotic environment will be limited. Given the low likelihood of occurrence of horizontal gene transfer and lack of adverse consequences if it were to occur, the import, processing, and food and feed use of MS11 x RF3 *B. napus* in the EU is not likely to pose any risk to human and animal health or the environmental.

Considering the low exposure and lack of hazard from horizontal gene transfer of the *barnase*, *barstar* and *bar* genes from MS11 x RF3 *B. napus* to micro-organisms resulting from the import, processing and all uses of MS11 x RF3 *B. napus*, the risk that this would result in adverse effects on human or animal health or the environment is negligible.

(d) Interactions between the genetically modified plant and target organisms

MS11 x RF3 *B. napus* has been developed to confer tolerance to glufosinate-ammonium, no target organisms are associated with this product, and therefore an assessment of the potential resistance development in target organisms resulting from the import, processing and food and feed use of MS11 x RF3 *B. napus* is not relevant for this application.

(e) Interactions of the genetically modified plant with non-target organisms

The scope of this application covers the import, processing and food and feed use of MS11 x RF3 *B. napus* in the EU, no deliberate release of viable plant material in the EU environment is expected. Therefore an assessment of potential direct effects of MS11 x RF3 *B. napus* on NTO populations is not relevant for this application. However, the assessment considers potential indirect adverse effects on NTO populations due to exposure through faeces of animals fed with MS11 x RF3 *B. napus*.

Exposure to faeces of animals fed with MS11 x RF3 *B. napus* would lead to very low levels of environmental exposure. The newly expressed proteins are expressed at low levels in seed and they are readily degraded by enzymatic activity in the gastro-intestinal tract of animals. Only minimal amounts of these proteins will be present in animal faeces. There would subsequently be further degradation of these proteins due to microbial processes. Exposure of soil and water environments to these proteins from disposal of animal wastes is likely to be very low and localized. Thus exposure of potentially sensitive NTOs (e.g. coprophagous Coleoptera species) to the MS11 x RF3 *B. napus* is likely to be very low and of no ecological relevance.

(f) Effects on human health

See point 9.

(g) Effects on animal health

See point 9.

(h) Effects on biogeochemical processes

Cultivation of MS11 x RF3 *B. napus* in the EU is not included in the scope of this application. Although environmental exposure could occur through the accidental spillage of MS11 x RF3 *B. napus*, or through manure or faeces of animals fed on MS11 x RF3 *B. napus* through organic matter or by-products from MS11 x RF3 *B. napus*, these routes of exposure would represent very low levels of exposure that would be limited spatially and temporally. It is highly unlikely that adverse effects on biogeochemical processes could occur. Therefore, an assessment of the impacts of MS11 x RF3 *B. napus* on

biogeochemical processes resulting from specific cultivation, management and harvesting techniques is not relevant given the scope of this application.

(i) Impacts of the specific cultivation, management and harvesting techniques

Cultivation of MS11 x RF3 *B. napus* in the EU is not included in the scope of this application. Therefore, an assessment of the impacts of specific cultivation, management and harvesting techniques of MS11 x RF3 *B. napus* is therefore not relevant given the scope of this application.

11.3. Potential interactions with the abiotic environment

Overall results of the comparative analysis of MS11 x RF3 *B. napus* with respect to its conventional counterpart indicate that observed differences in composition and agronomic and phenotypic characteristics fell within the range of natural variability for oilseed rape with a history of safe use. Therefore, there is no evidence that this oilseed rape would be any different from conventional oilseed rape with regard to its baseline interactions with the abiotic environment.

In addition, because this application is for import, processing and all uses as any other oilseed rape in the EU, but excluding cultivation, interactions of MS11 x RF3 *B. napus* with the environment will be limited.

11.4. Risk characterisation

The ERA has been conducted following the requirements and methodology described in the EFSA Guidance documents. The baseline considered for this risk assessment is the use of conventional oilseed rape in the EU, applying the concept of “familiarity”, where the fact that oilseed rape is a common crop in the EU, previously used as food and feed for centuries and considered safe for human and animal health and the environment.

A comparative safety assessment has been conducted using a weight-of-evidence approach, considering molecular characterization data as well as expression, compositional and agronomic comparisons between the product and its conventional counterpart. This assessment has been used to establish whether unintended changes in the GM plant have occurred as a result of the combination of the single events or interactions between the gene products. The results of this comparative safety assessment demonstrated that the only differences of biological relevance identified between MS11 x RF3 *B. napus* and the conventional counterpart are the intended traits. Despite the large number of parameters compared, no unintended differences of biological relevance were found. Thus the exposure and hazard assessment conducted for the single events have been used to support the ERA of MS11 x RF3 *B. napus*.

An assessment whether MS11 x RF3 *B. napus* will be more persistent than the conventional crop in agricultural habitats or more invasive in natural habitats has been conducted. The results of this assessment allowed the conclusion that the risk that the import, processing or food and feed use of MS11 x RF3 *B. napus* in the EU will not result in harm to sustainable agricultural production or biodiversity as a result of changes in persistence or invasiveness compared with the conventional crop is negligible.

An assessment whether the new genes present in MS11 x RF3 *B. napus* could be transferred into micro-organisms and become integrated into their genome leading to adverse effects in human and animal health or the environment has been performed. The conclusion from this assessment was that it is very unlikely that these genes would become established in the genome of micro-organisms in the environment or human and

animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected.

Potential interactions with target and non-target organisms that could lead to harmful environmental effects have also been assessed. The conclusion from these assessments is that adverse effects on sustainable agricultural production or biodiversity due to adverse effects on populations of NTOs as resulting from the import, processing or food and feed use MS11 x RF3 *B. napus* will be negligible.

No assessment of adverse environmental effects due to changes in management practices or effects on biogeochemical processes has been performed since cultivation of MS11 x RF3 *B. napus* is not within the scope of this application.

Finally, risks associated with the import, processing and food and feed use of MS11 x RF3 *B. napus* in the EU on human and animal health have been assessed. The conclusion from this assessment was that food and feed derived from MS11 x RF3 *B. napus* is as safe for humans and animal consumption as food and feed derived from the conventional crop.

In summary the import, processing and food and feed use of MS11 x RF3 *B. napus* in the EU will pose negligible risk to human and animal health or the environment. The uncertainties associated with this risk characterisation are very low and no long-term adverse environmental effects are expected.

12. ENVIRONMENTAL MONITORING PLAN

(a) General (risk assessment, background information)

As required by Article 5(5)(b) and 17(5)(b) of Regulation (EC) No 1829/2003 the proposed Post-Market Environmental Monitoring (PMEM) plan for MS11 x RF3 *B. napus* has been developed according to the principles and objectives outlined in Annex VII of Directive 2001/18/EC and Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC. The PMEM also takes into account the Scientific Opinion on guidance on the Post-Market Environmental Monitoring of genetically modified plants

(b) Interplay between environmental risk assessment and monitoring

An environmental risk assessment (e.r.a.) was carried out for MS11 x RF3 *B. napus* according to the principles laid down in Annex II to Directive 2001/18/EC and Decision 2002/623/EC establishing guidance notes supplementing Annex II to Directive 2001/18/EC. The scientific evaluation of the characteristics of MS11 x RF3 *B. napus* in the e.r.a. has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of MS11 x RF3 *B. napus*.

(c) Case-specific genetically modified plant monitoring (approach, strategy, method and analysis)

The scientific evaluation of the characteristics of MS11 x RF3 *B. napus* in the ERA has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of MS11 x RF3 *B. napus*. It is therefore considered that there is no need for case-specific monitoring.

(d) General surveillance of the impact of the genetically modified plant (approach, strategy, method and analysis)

In accordance with Council Decision 2002/811/EC, general surveillance is not based on a particular hypothesis and it should be used to identify the occurrence of unanticipated adverse effects of the viable Genetically Modified Organism (GMO) or its use for human and animal health or the environment that were not predicted in the ERA.

The scope of this application is the authorisation of MS11 x RF3 *B. napus* for food and feed uses, import and processing. The scope of the application does not include authorisation for the cultivation of MS11 x RF3 *B. napus* seed products. Therefore, exposure to the environment will be limited to unintended release of MS11 x RF3 *B. napus*, which could occur for example via substantial losses during loading/unloading of the viable commodity including MS11 x RF3 *B. napus* destined for processing into animal feed or human food products. Exposure can be controlled by clean up measures and the application of current practices used for the control of any adventitious oilseed rape plants, such as manual or mechanical removal and the application of herbicides (with the exception of glufosinate ammonium herbicide).

However and in order to safeguard against any adverse effects on human and animal health or the environment that were not anticipated in the ERA, general surveillance on MS11 x RF3 *B. napus* will be undertaken for the duration of the authorisation. The general surveillance will take into consideration, and be proportionate to, the extent of imports of MS11 x RF3 *B. napus* and use thereof in the Member States.

In order to increase the possibility of detecting any unanticipated adverse effects, a monitoring system will be used, which involves the authorisation holder and operators handling and using viable MS11 x RF3 *B. napus*. The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable MS11 x RF3 *B. napus*.

(e) Reporting the results of monitoring

In accordance with Regulation (EC) No 1829/2003, the authorisation holder is responsible to inform the European Commission of the results of the general surveillance.

If information that confirms an adverse effect of MS11 x RF3 *B. napus* and that alters the existing risk assessment becomes available, the authorisation holder will immediately investigate and inform the European Commission. The authorisation holder, in collaboration with the European Commission and based on a scientific evaluation of the potential consequences of the observed adverse effect, will define and implement management measures to protect human and animal health or the environment, as necessary. It is important that the remedial action is proportionate to the significance of the confirmed effect.

The authorisation holder will submit an annual monitoring report including results of the general surveillance in accordance with the conditions of the authorisation. The report will contain information on unanticipated adverse effects, if any, that have arisen from handling and use of viable MS11 x RF3 *B. napus*.

The report will include a scientific evaluation of the confirmed adverse effect, a conclusion of the safety of MS11 x RF3 *B. napus* and, as appropriate, the measures that were taken to ensure the safety of human and animal health or the environment.

The report will also clearly state which parts of the provided information are considered to be confidential, together with a verifiable justification for confidentiality in accordance with

Article 30 of Regulation (EC) No 1829/2003. Confidential parts of such report shall be submitted in separate documents.

13. DETECTION AND IDENTIFICATION TECHNIQUES FOR THE GENETICALLY MODIFIED PLANT

The detection method for MS11 x RF3 *B. napus* was sent to the Community Reference Laboratory (CRL) of the Joint Research Centre of the European Commission (EC-JRC) for the purposes of experimental testing and validation in the frame of the food and feed application of MS11 x RF3 *B. napus*. Appropriate control samples were also made available to the JRC-CRL.

14. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GENETICALLY MODIFIED PLANT (FOR ENVIRONMENTAL SAFETY ASPECTS)

14.1. History of previous releases of the genetically modified plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

(a) Notification number

There is no history of field release of MS11 x RF3 *B. napus* in the EU.

(b) Conclusions of post-release monitoring

Not applicable.

(c) Results of the release in respect to any risk to human health and the environment, submitted to the Competent Authority in accordance with Article 10 of Directive 2001/18/EC)

Not applicable.

14.2. History of previous releases of the genetically modified plant carried out outside the Union by the same notifier

(a) Release country

MS11 x RF3 *B. napus* has been tested in Canada and the USA (and Chile in a contra season program).

(b) Authority overseeing the release

Canada: Canadian Food Inspection Agency; U.S.: U.S. Department of Agriculture; Chile: SAG

(c) Release site

Canada and U.S.: multiple major canola-growing provinces, states and regions respectively; northern Chile.

(d) Aim of the release

Regulatory trials, testing of efficacy, yield and product development, and contra season program in Chile

(e) Duration of the release

MS11 x RF3 has been tested in Canada for 10 years.

(f) Aim of post-releases monitoring

Volunteer assessment.

(g) Duration of post-releases monitoring

The CFIA confined permits require 3 years of post-trial monitoring.

(h) Conclusions of post-release monitoring

Oilseed rape volunteers are sometimes observed since oilseed rape has secondary dormancy. If volunteers occur, the practice is to eliminate them manually or chemically to prevent occurrence in subsequent crops.

(i) Results of the release in respect to any risk to human health and the environment

Field-testing provided no evidence that MS11 x RF3 *B. napus* would be the cause of any adverse effects to human health or to the environment.